

## AMENDED CLAIM SET

1. (CURRENTLY AMENDED) A method of loading ~~[[a]]~~ lipid-like vesicles ~~having a membrane permeable to a~~ with a chemical species ~~to be loaded from a loading solution wherein the concentration of the thus loaded chemical species within the vesicle [[is]] is greater than the concentration of the chemical species in the loading solution and the loaded chemical species can be substantially maintained within the vesicle for at least one-quarter hour following loading,~~ comprising:

forming lipid-like vesicles in a solution comprising an acidic buffer if the chemical species to be loaded is basic or a basic buffer if the chemical species to be loaded is acidic; wherein:

membranes of the formed lipid-like vesicles are impermeable to the buffer;  
adjusting the pH of the solution exterior to the membranes of the lipid-like vesicles to a basic pH if the chemical species to be loaded is basic or to an acidic pH if the chemical species to be loaded is acidic;

adding a basic chemical species to the adjusted basic exterior solution or an acidic chemical species to the adjusted acidic exterior solution;

loading the chemical species into the vesicle; and

adjusting the exterior solution to a physiologically benign pH; wherein:

the chemical species is substantially maintained in the vesicle for at least one quarter hour after the adjustment of the exterior solution.

2. (CURRENTLY AMENDED) A method of loading lipid-like vesicles having a membrane permeable to a chemical species to be loaded and for substantially maintaining the loaded chemical species within the vesicle for at least one-quarter hour following loading by inducing a pH gradient across the membrane, comprising:

(1) incorporating within the vesicle a buffer solution buffered to a selected acid or alkaline pH having a selected molarity and at least one selected pKa approximately equal to the selected buffer pH, the membrane being substantially impermeable to the buffer for at least one-quarter hour following loading of the chemical species;

(2) positioning the vesicles in a bulk solution having a selected pH; and  
(3) providing the bulk solution with a chemical species having one or more selected acid pH responsive groups if the buffer is alkaline or one or more basic pH responsive groups if the buffer is acidic wherein the pH of the bulk solution is at least ~~respectively~~ 0.5, 0.3 or 0.2 of a pH unit higher than the pH of the buffer if the buffer is acidic and the chemical species has ~~respectively~~ one, two, or three or more basic pH responsive groups, or the pH of the bulk solution is at least ~~respectively~~ 0.5, 0.3 or 0.2 of a pH unit lower than the pH of the buffer if the buffer is basic and the chemical species has ~~respectively~~ one, two or three or more acid pH responsive groups, the pH responsive groups of the chemical species having one or more acid pH responsive groups have a pKa that is generally lower than or equal to the pH of the bulk solution and generally higher than or equal to 3.5 and the pH responsive groups of the chemical species having one or more basic pH responsive groups have a pKa that is generally higher than or equal to the pH of the bulk solution and generally lower than or equal ~~tell~~ to 11.

3. (CURRENTLY AMENDED)                      A method according to claim 2 wherein the pH responsive group or groups are acid pH responsive groups and the buffer has a pKa ~~in the range~~ of about 10.

4. (CURRENTLY AMENDED)                      A method according to claim 3 wherein the chemical species has a pKa ~~from~~ of about 4-7.

5. (PREVIOUSLY PRESENTED)                      A method according to claim 4 wherein the pH responsive group is a carboxyl group.

6. (CURRENTLY AMENDED)                      A method according to claim 2 wherein the pH responsive group or groups are basic pH responsive groups, and the buffer has a pKa in the range of about ~~[[S]]~~ 5.

7. (PREVIOUSLY PRESENTED) A method according to claim 6 wherein the chemical species has a pKa from about 7-10.

8. (PREVIOUSLY PRESENTED) A method according to claim 7 wherein the pH responsive group is an amino group.

9. (PREVIOUSLY PRESENTED) A method according to claim 8 wherein the chemical species is an amine.

10. (PREVIOUSLY PRESENTED) A method according to claim 2 wherein the vesicle is prepared in the buffer and incorporates the buffer via mixing and sonication.

11. (CURRENTLY AMENDED) A method according to claim 2 wherein the pH of the bulk solution is ~~from~~ about 7.0 to about 7.8.

12. (CURRENTLY AMENDED) A method according to claim 11 wherein the pH of the bulk solution is ~~but~~ about 7.4.

13. (WITHDRAWN AND CURRENTLY AMENDED) ~~Vesicles prepared according to~~ The method of claim 2 wherein the chemical species is a drug.

14. (WITHDRAWN) A pharmaceutical preparation for administration in vivo to an animal comprising lipid-like vesicles prepared according to claim 1 wherein said chemical species is a drug.

15. (WITHDRAWN AND CURRENTLY AMENDED) A pharmaceutical preparation for parenteral administration in vivo to an animal comprising ~~liposomes~~ lipid-like vesicles prepared according to claim 2 wherein said chemical species is a drug, the osmolarity of the buffer is within the physiological range of the animal, the vesicles are

suspended for administration in the bulk solution, and the pH of the bulk solution is physiologically benign.

16. (CURRENTLY AMENDED) A kit for loading lipid-like vesicles having a membrane permeable to the chemical species to be loaded comprising:

- (1) a first compartment having a first solution having membranous lipid-like vesicles incorporating a buffer buffered to a selected acid or basic pH, the buffer having at least one selected pKa approximately equal to the selected buffer pH and a selected molarity and being substantially impermeable to the vesicle's membrane for at least one-quarter hour following loading of the chemical species and the first solution having a selected pH such that the stability of the vesicle and its buffer can be maintained for a period of at least one week at 4°C ~~4 °C~~.
- (2) a second compartment, separate from the first compartment, having a second solution having a selected pH;
- (3) a chemical species permeable to the vesicle having a selected pKa and one or more selected acid pH responsive groups if the buffer is basic or one or more basic pH responsive groups if the buffer is acidic, the chemical species being initially present in a selected one of two solutions with the second solution having a ~~[[ps]]~~ pH such that a mixture of the first and second solutions would have a pH ~~respectively~~ of at least 0.5, 0.3, or 0.2 of a pH unit higher than the pH of the buffer if the buffer is acidic and the chemical species has ~~respectively~~ one, two, or three or more basic pH responsive groups ~~or a pH at least, respectively,~~ 0.5, 0.3 or 0.2 of a pH unit lower than the pH of the buffer if the buffer is basic and the chemical species has ~~respectively~~ one, two or three or more acid pH responsive groups, the pH responsive groups of the chemical species having one or more acid pH responsive groups have a pKa that is generally lower than or equal to the pH of the mixture of the first and second solution and generally higher than or equal to 3.5 and the pH responsive groups of the chemical species having one or more basic pH responsive groups have a pKa that is generally higher than or equal to the pH of the mixture of the first and second solutions and generally lower than or equal to 11.

17. (PREVIOUSLY PRESENTED) A kit as set forth in claim 16 wherein said chemical species is a drug.

18. (CURRENTLY AMENDED) A kit ~~[[an]]~~ as set forth in claim 17 wherein the mixture will have a pH that is physiologically benign in regard to the blood of a mammal.

19. (CURRENTLY AMENDED) A kit as set forth in claim 18 further ~~including~~ comprising means for parenterally delivering the mixture to a mammal in vivo.

20. (CURRENTLY AMENDED) A kit for loading lipid-like vesicles having a membrane permeable to an acid or basic chemical species to be loaded comprising:

- (1) a first compartment having a first solution having membranous ~~lipid~~ lipid-like vesicles incorporating a buffer buffered to a selected basic pH if the chemical species to be loaded is an acid or acid pH if the species in a base, the buffer having a selected pKa and a selected molarity, the membrane being substantially impermeable to the buffer for at least one-quarter hour following loading of the chemical species, the first solution having a selected pH such that the stability of the vesicle and its buffer will be maintained for a period of at least one week at ~~[[4° C.]]~~ 4 °C;
- (2) a second separate compartment having a first substance which when combined with the first solution will adjust the pH of the first solution so as to provide a predetermined pH gradient between the buffer within the vesicle and the pH adjusted first solution; and
- (3) a third separate compartment having a second substance which when combined with the pH adjusted first solution will further change the pH of said solution to a physiologically benign value with regard to the blood of a mammal.

21. (CURRENTLY AMENDED) A kit as set forth in claim 20 further ~~including~~ comprising a selected chemical species.

22. (PREVIOUSLY PRESENTED)      A kit as set forth in claim 21 wherein the selected chemical species is a drug.

23. (PREVIOUSLY PRESENTED)      A kit as set forth in claim 22 further including a means for parentally delivering the vesicle solution having the physiologically benign adjusted pH to a mammal in vivo.

24. (WITHDRAWN)      A method of detoxifying an animal suffering from an overdose of a chemical species with basic pH responsive groups comprising injecting the animal with a solution having a physiologically benign pH with respect to the animal, the solution having large volumes of liposomes having a buffer solution buffered to a pH generally lower than or equal to 5.4 and the buffer having at least one selected pKa and a selected molarity within the physiological range of the animal the liposomes being substantially impermeable to the buffer for at least one hour after injection.

25. (WITHDRAWN)      A method for detoxifying an animal suffering from an overdose of a chemical species with acid pH responsive groups the chemical species being permeable to liposomes comprising: injecting the animal with a solution having a physiologically benign pH with respect to the animal, the solution having large volumes of liposomes having a buffer solution buffered to a pH generally higher than or equal to 9.4 and having a selected solarity and selected pKa, the liposomes being substantially impermeable to the buffer for at least one hour after injection.

26. (WITHDRAWN)      A method of loading lipid-like vesicles having a membrane permeable to a chemical species to be loaded and substantially maintaining the loaded chemical species by inducing a pH gradient across the membrane within the vesicle for at least one-quarter hour following loading, comprising:

- (1) incorporating within the vesicle a buffer solution buffered to a selected acid or alkaline pH and having a selected molarity and at least one selected pKa, the

membrane being substantially impermeable to the buffer for at least one-quarter hour following loading of the chemical species;

- (2) positioning the vesicles in a bulk solution having a selected pH of either 0.5 to 3 pH units lower or pH units higher than the pH of the buffer thereby establishing a transmembrane electrical potential and a positive charge inside the vesicle if the pH outside the vesicle is more acid than inside or a negative charge inside the cell if the pH outside the call is more basic than inside;
- (3) providing in the bulk solution a chemical species having hydrophobic negatively-charged ions if the membrane charge within the vesicle is positive or hydrophobic positively charged ions if the membrane charge within the vesicle is negative.